and anti-G3 quantitative Western blot showed aggrecan fragment patterns for individual subjects, allowing a detailed comparison of aggrecan proteolysis in different human joint diseases and disease stages. SF aggrecan fragment patters differed significantly between different human joint diseases, suggesting differences in protease activity and or structure of the cartilage matrix substrate. Use of additional antibodies will allow quantification of other aggrecan fragments. The method can help identify new OA biomarkers. It is also suitable for in vitro screening of protease inhibitors and in early development of disease modifying therapy of human joint diseases.

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BIOLOGICAL VARIABILITY OF BIOCHEMICAL MARKERS OF BONE, CARTILAGE AND SYNOVIAL METABOLISM IN PATIENTS WITH KNEE OSTEOARTHRITIS: EFFECT OF FOOD INTAKE

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Purpose: Biochemical markers (BM) may be useful to assess disease progression and efficacy of treatments in osteoarthritis (OA). The clinical utility of BMs depends on two components: 1) their sensitivity to detect changes with disease or treatment and 2) their biological variability which may be affected by food intake. The aim of this study was to evaluate the effect of food intake on the concentration and the between day intra-subject variability of BMs in untreated patients with knee OA and in healthy subjects of similar age.

Methods: We recruited 22 patients (13 women, 9 men, mean age 58 yr) with radiographic knee OA (12 with Kellgren-Lawrence [KL] score 2 and 10 with KL score 3) and 10 healthy subjects (7 women, 3 men, mean age: 47 yr) with no radiographic OA at the knees, hips and spine. No subject was treated with any investigational OA drugs, estrogen or bisphosphonate within the previous 3-6 months and during the 14 days study period. When receiving NSAIDs and/or dietary supplements, no change in therapy was permitted during the study. For each subject and at each of the 3 visits (day 1, day 7 and day 14), morning blood samples were collected at two time points: fasting (F) at 7.45 before an 8.00 breakfast and non-fasting (NF) at 10.00, 2 hours after breakfast. Second morning void urine was also collected at each 3 visits. The following serum (S) and urine (U) BMs were measured: S-CTX-I, a BM of bone resorption, S-COMP, a BM of cartilage turnover, S-PIIANP, a BM of cartilage synthesis, hyaluronic acid (S-HA), a BM of synovitis, U-CTX-II, a BM of cartilage degradation and U-Glc-Gal-PYD, a BM of synovial tissue metabolism. For S-BMs, the difference in levels between NF and F state was calculated and the intra-subject variability was estimated by mixed model ANOVA.

Results: Patients with knee OA had higher median levels of S-COMP (+30%, p=0.02 and +24%, p=0.05 for F and NF samples, respectively), S-HA (+48%, p=0.02 and +13%, p=0.12 for F and NF samples) and CTX-II (+108%, $p<0.01$) than healthy controls. Patients with knee OA KL score 3 had higher F-levels of S-CTX-I (+64%, p=0.02), S-COMP (+37%, p=0.02), S-HA (+68%, p=0.03), U-CTX-II (+151%, $p<0.001$) and U-Glc-Gal-PYD (+53%, p=0.01) than patients with KL-2. When measured on NF samples, the difference between patients with KL-2 and KL-3 was not anymore significant for S-CTX-I and S-HA. The table shows the difference of S-BM levels in NF compared with F state as a percentage (% NF-F) and the intra subject coefficient of variation (CVi%) in all subjects, in patients with knee OA, and in healthy controls and in F (CVi-F) and NF (CVi-NF) conditions. For urinary markers, the intra-subject variability in all subjects, knee OA patients and healthy controls were respectively 23.1%, 19.6% and 30.5% for U-CTX-II and 21.1%, 22.3% and 16.4% for U-Glc-Gal-PYD.

Conclusions: Food intake has a marked influence on the levels of S-CTX-I and S-HA and increases variability of S-CTX-I, but has small effects on S-COMP and S-PIIANP. The association of S-BMs with OA was higher when measured on F than NF samples. Standardized collection of fasting sample is recommended for measuring BM in OA.

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PATIENTS WITH RHEUMATOID ARTHRITIS HAVE AN ALTERED AGGRECAN PROFILE IN SERUM

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Purpose: Rheumatoid Arthritis (RA) is a chronic auto-immune disease with aggressive and extensive articular cartilage destruction. Aggrecan is the major proteoglycan of articular cartilage, and is composed of three globular domains (G1-G3). Aggrecan depletion, mediated by aggrecanases is one of the first signs of early cartilage erosion. We investigated, whether measurement of aggrecan and fragments thereof in serum, could be used as biomarkers for joint-disease in RA patients.

Methods: For the verification of release of intact aggrecan and degradation fragments, we used catabolically stimulated human cartilage explants from a late stage 42 year old female patient with osteoarthritis (OA). Pieces of cartilage (12-16 mg) were placed in 96 well plates in 4-replicates and incubated for 21 days at 37°C with 5% CO_2 and shaking (50 rpm). Serum-free D-MEM medium was used. Explants were incubated with either medium alone, or 10 and 20 ng/ml of the pro-inflammatory cytokines OSM/TNFα respectively. As negative control, cartilage was placed in cryo-tubes, frozen in liquid N_2 and thawed at 37°C in water-bath for three repeated freeze-thaw cycles. The explant culture medium was replaced every 3rd day for 21 days.

The study co-hort consisted of 108 healthy individuals and 38 patients diagnosed with RA (61.3±19.3 years, 71.05 ±14.5 kg, 4.7±1.6 disease activity score). Aggrecan levels were investigated by, 1) ^{374}ARGSVI-G2 sandwich assay measuring aggrecanase-mediated aggrecan degradation, or 2) G1/G2 sandwich assay, believed to account for the total turnover of aggrecan. We further characterised serum samples by western blots,

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	S-CTX-I			S-COMP			S-PIIANP			S-HA		
	% NF-F	CVi-F	CVi-NF	%NF-F	CVi -F	CVi -NF	% NF-F	CVi -F	CVi -NF	% NF-F	CVi -F	CVi-NF
All	-52%**	12.9	28.6	0.1%	8.8	8.8	-2.0%	19.7	25.1	+33%**	34.3	34.2
Knee OA	-52%**	12.5	29.8	-0.1%	8.5	8.9	-1.9%	19.6	25.7	+30%**	36.1	37.0
Healthy	-51%*	13.0	23.9	0.4%	9.2	8.0	-8.9%	20.4	20.6	+45%*	19.7	22.0

*$p<0.01$, **$p<0.001$.

through 1) the similar antibody used in the G1/G2 ELISA assay, F-78, binding to a repetitive epitope exposed twice on G1 and G2 or, 2) by BC-3 detecting the aggrecanase-generated N-terminal ^{374}ARGSVI sequence.
Results: Induction of cartilage degradation *ex vivo*, resulted in 10 fold elevated aggrecanase fragments (^{374}ARGSVI-G2) (P=0.02) and 9 fold increased total aggrecan release (G1/G2) (P=0.02), compared to that of vehicle control. Total aggrecan levels in RA patients were significantly decreased from 824.8±31 ng/ml in healthy controls to 570.5±30 ng/ml, corresponding to an approximate 31% decrease ($P<0.0001$). Western blot analysis for total aggrecan showed one strong band detected at 10 kDa, and weaker bands at 25 and 45 kDa in both healthy controls and RA patients. With regards to aggrecanase activity, only one strong band in RA patients of 45 kDa was detected.
Conclusions: This study is the first to characterize different aggrecan fragments in human serum. We clearly demonstrate that total aggrecan levels are lowered in RA patients. In alignment, RA patients had highly elevated aggrecanase-mediated aggrecan degradation, providing a rationale for the decreased aggrecan levels. These data may provide a new diagnostic tool for monitoring joint-destruction in RA patients, and allow for early assessment of response to treatment prior to that of traditional radiographic techniques.

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CHARACTERIZATION OF METALLOPROTEINASE CLEAVAGE PRODUCTS OF HUMAN ARTICULAR CARTILAGE

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Purpose: The present study measures the effects of metalloproteases on human articular cartilage. Supernatants from digestions with these enzymes were analyzed via proteomics methods to characterize post-translational modifications of cartilage peptides, to determine specific cartilage protein cleavage sites of different metalloproteases to better understand their selectivities, and to measure release of peptides that may serve as useful biomarkers of cartilage degradation. The metalloproteases utilized in this study have been reported to be overexpressed and active in arthritic cartilage, so determination of their substrates and products may lead to a better understanding of their potential role in disease and to identification of new targets and biomarkers of cartilage degradation.
Methods: Human articular cartilage was digested by addition of exogenous metalloproteases, including MMP-2, -3, -8, -9, -12, -13, ADAMTS-4, and ADAMTS-5, and proteolyzed peptide products were identified by proteomics methods using mass spectrometry.
Results: Complete sequences of peptides, including N- and C-termini and post-translational modifications were determined. Abundant peptides originating from collagen types I, II, and III, biglycan, prolargin, fibromodulin, fibronectin, decorin, cartilage oligomeric matrix protein, cartilage intermediate layer protein, megakaryocyte stimulating factor, mimecan, aggrecan, and lumican were identified after metalloprotease digestion. Specific collagen type II peptide biomarkers, including those containing the 3/4 – 1/4 cleavage site and those containing the Helix-II and CTX-II domains, were observed after release by selected proteases.
Conclusions: this study identifies many of the most abundant metalloprotease cleavage products of human articular cartilage. The list of peptides generated by these digestions provides insights into the broad number of substrates susceptible to specific metalloprotease proteolysis in diseased cartilage. Many of these peptides are potential biomarkers of arthritis or of metalloprotease activity in articular cartilage. Further evaluation of many of these peptides as biomarkers might be achieved by quantitation in synovial fluid, blood, or urine.

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EFFECTS OF EXERCISE AND OSTEOCHONDRAL INJURY ON CTX II CONCENTRATIONS IN EQUINE SYNOVIAL FLUID AND SERUM

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Purpose: Collagen type II is the predominant collagen in articular cartilage and is highly specific for this tissue. A biomarker of crosslinked C-telopeptide fragments of type II collagen (CTX II) has been used to assess severity and progression of cartilage degradation in humans. The objectives of the current study were to evaluate the effects of exercise and osteochondral (OC) injury on concentrations of CTX II fragments in synovial fluid (SF) and serum of horses.
Methods: SF and serum samples were taken from 3 groups of Thoroughbred racehorses: (1) rested horses (n=40), (2) exercised horses: group 1 horses had the same joints sampled after 4-7 months of race training (n=40) and (3) OC injured horses: racehorses that had arthroscopic surgery for removal of OC fragments resulting from racing injury (n=44). From group 1 and 2 horses, SF was obtained from 20 metacarpophalangeal (MCP),

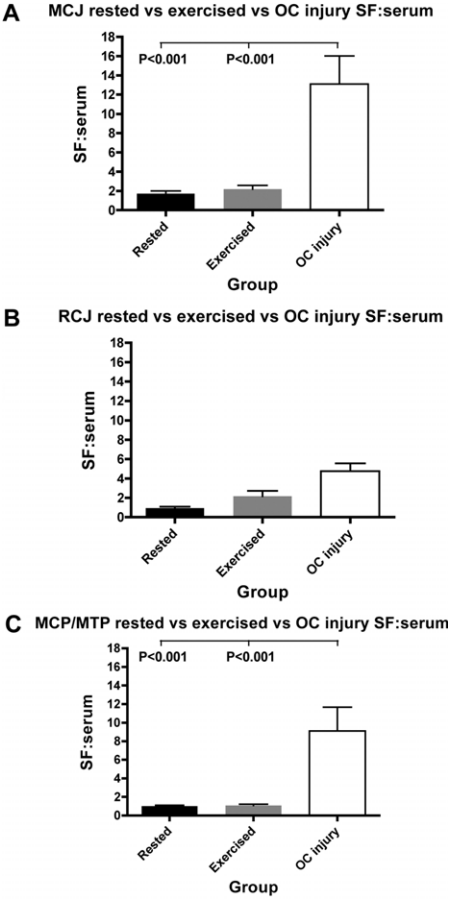

Figure 1. Comparison of SF to serum CTX II concentrations (SF:serum) in rested, exercised, and OC injured horses. A. MCJ - middle carpal joint; B. RCJ - radiocarpal joint; C. MCP/MTP - metacarpophalangeal/metatarsophalangeal joint.